

FINAL REPORT

STUDY OF

IN SITU VOLATILIZATION OF SELENIUM

II. EVAPORATION PONDS

EXECUTIVE SUMMARY

September 1989

**Prepared under Contract
for the Federal-State
San Joaquin Valley Drainage Program**

This report presents the results of a study conducted for the Federal-State Interagency San Joaquin Valley Drainage Program. The purpose of the report is to provide the Drainage Program agencies with information for consideration in developing alternatives for agricultural drainage water management. Publication of any findings or recommendations in this report should not be construed as representing the concurrence of the Program agencies. Also, mention of trade names or commercial products does not constitute agency endorsement or recommendation.

The San Joaquin Valley Drainage Program was established in mid-1984 as a cooperative effort of the U.S. Bureau of Reclamation, U.S. Fish and Wildlife Service, U.S. Geological Survey, California Department of Fish and Game, and California Department of Water Resources. The purposes of the Program are to investigate the problems associated with the drainage of irrigated agricultural lands in the San Joaquin Valley and to formulate, evaluate, and recommend alternatives for the immediate and long-term management of those problems. Consistent with these purposes, Program objectives address the following key areas: (1) Public health, (2) surface- and ground-water resources, (3) agricultural productivity, and (4) fish and wildlife resources.

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Contract No. 7-FC-20-05110

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High selenium levels in agricultural drainage water and soil are a major problem for California farmers, environmentalists and state and federal legislators. Selenium is also a widespread contaminant throughout the U.S. including Arizona, Colorado, Montana, Nevada, New Mexico, South Dakota, Utah and Wyoming. Wildlife aberrations and toxicities are becoming apparent and solutions are being sought to remediate this problem. The agricultural drainage water in areas of the San Joaquin Valley is extremely saline and high in selenium. This saline, alkaline water is disposed of into large evaporation ponds. These ponds range in size between a few acres to over 750 acres. Each grower must sacrifice roughly 10 to 15% of his land to an evaporation pond facility in order to continue farming in certain areas of the San Joaquin Valley. It is estimated that over 7 million tons of salts including selenium are accumulating yearly in the farmlands of the San Joaquin Valley through irrigation practices. According to a 1989 U.C. Salinity/Drainage Task Force panel (a group of experts who were asked by the state and federal governments to assess and conduct research on the farming problems in the Valley) there are few preventative methods available on selenium accumulation. Biological deselenification may be a novel but practical method to deal with the yearly increase in selenium concentrations in evaporation ponds.

Selenium levels in some agricultural drainage evaporation ponds within the San Joaquin Valley are continuing to increase over time.

Many sites have levels that are well above the 1987 California State Water Resources Control Board water quality recommendation of 5 ppb Se for drainage water suitable for discharge into rivers.

The technology of biological deselenification is still in its infancy. Over the last two years, our laboratory has focused on research that dramatically accelerates this naturally occurring transformation. This microbial reaction converts toxic selenium compounds present in the water into a non-hazardous, gaseous form, dimethylselenide (DMSe) which is liberated into the atmosphere. DMSe is non-hazardous to rats. The scientific terms for this process include: selenium "biomethylation", "alkylation", "transmethylation", "volatilization", and "deselenification". Although the organisms responsible for biomethylation are microscopic, they are able to multiply relatively fast in the presence of nutrients and make a substantial contribution to water deselenification in a relatively short period of time.

One objective we wanted to assess at the beginning of this project was whether selenium biomethylation occurs in evaporation pond water. While we already had some experience with selenium methylation in soils, there was some skepticism among fellow scientists as to whether it would occur directly in pond water without the influences of the sediment. Despite the harsh, saline conditions found in evaporation pond water, it was quickly discovered that biomethylation did indeed occur, albeit at very low levels, in water collected from these ponds. We were able

to confirm this by capturing the end-product, DMSe, and analyzing it using gas chromatography and mass spectroscopy. Bacteria and fungi were isolated which are resistant to selenium concentrations between 1 and 100 ppm. One particular isolate, Alternaria alternata, was found to produce large quantities of DMSe. This fungus was particularly efficient at methylating the common forms of selenium found in the water, selenite and selenate, even at extremely high concentrations and was stimulated by the addition of cofactors (chemicals which facilitate biological reactions) such as methionine and methyl cobalamine.

No selenium alkylation occurred in sterilized pond water. This confirmed that the process is a biological rather than a chemical reaction. Treatments such as increasing the temperature to 35°C, adding a fungal inoculum as well as carbon amendments consisting of simple sugars, complex carbohydrates, and amino acids increased biomethylation slightly. Not until proteins were added did we uncover an exciting discovery. Albumen (a protein found in the white of egg), casein (a milk protein) and gluten (a wheat protein) dramatically increased DMSe evolution by pond water at all of the concentrations tested. After 43 days of incubation, albumen, casein and gluten (2 g carbon L⁻¹) caused a 23%, 41% and 10% selenium loss from the inventory, respectively.

Encouraged by the discovery that proteins dramatically stimulated volatilization, other organic amendments were tested including saccharides (mono-, poly- and acidic), alcohols, fats and oils. None of these compounds

were used by the aquatic microorganisms to fuel the methylation reaction. We therefore decided to further investigate the stimulatory components of the milk-protein, casein and characterize its effects on Se methylation in pond water. Casein was heat-treated, boiled with acid, incubated with enzymes, purified, dialyzed, separated into its building blocks (proteins, peptides, amino acids, nitrogen) and minerals were removed. It was concluded that the active ingredient of casein is likely to be a peptide mixture. Casein did not appear to increase selenium methylation through any concurrent changes influencing the physicochemical and chemical characteristics of the water such as altered acidity/alkalinity, mineral content or chelation (binding) of pond water salts.

Our primary objective was to find sources of economical, readily available and unwanted protein/peptide sources that could be used in a water treatment process. All of the following by-products were good sources of proteins and stimulatory to the deselenification process: cottonseed and soybean meals (agricultural industry), cheese whey, whey protein (dairy industry by-products) and yeast sludge (food and beverage, e.g., beer industry).

Investigations with seleniferous pond water using microscopy techniques, antibiotic treatments, and microbiological counting techniques indicated that bacteria are the principal selenium methylating organisms in evaporation pond water. The addition of casein (4 g L^{-1}) to pond water not only stimulated biomethylation 25-fold but also increased the number of bacteria within the water by 1000-fold. We therefore

believe that proteins such as casein are important because of their nutritional value to the bacteria. The bacterial populations present in the drainage water are well adapted to their environment and resistant to a number of toxic trace elements including arsenic, boron, chromium, lead, molybdenum, selenium, silver and uranium naturally present in the pond water.

Optimum conditions for the protein-fuelled reaction were observed in a well-mixed, aerobic system. Growth attachment sites in the form of sand and glass beads were unnecessary for the successful multiplication and growth of the bacteria. The addition of trace amounts of reaction cofactors (methyl donors and reducing agents) further enhanced the methylation reaction in the water. The two major inorganic aquatic water species, selenite and selenate, were methylated equally well in the presence of protein. We found that methylation increased at lower water selenium concentrations. Artificially spiking the water with higher concentrations of selenium was not inhibitory. Dilution of the pond water with deionized water did not increase biomethylation despite the decrease in selenium, implying that selenium concentration, bacterial numbers and nutrients are far more crucial limitations to volatilization than high salinity. Another potential limitation to the process was high concentrations of nitrates and nitrites but the inhibitory concentrations were in excess of the natural levels of nitrogenous compounds found in evaporation pond water. Further increases in sulfates, a salt already present in extremely high concentrations in pond water, had no effect.

Field experiments were conducted directly in the evaporation ponds. We found that a single casein amendment (0.2 g C L^{-1}) caused a 38% selenium loss from the inventory of a San Joaquin Valley evaporation pond in a 142-day period.

Future studies should focus on successfully developing a deselenification water treatment process using the information collected from our laboratory and field studies. Further research is needed to develop various engineering techniques that are compatible with the previously researched optimum variables and also to continue screening economical nutrient sources which are needed to fuel the reaction. While the emphasis should be on environmental engineering it would also be of interest to pursue the genetic implications of biomethylation. Further field work is needed using continuous flow systems. We conclude that the acceleration of deselenification in evaporation pond water through the use of economical, available protein sources is a promising and novel bioremediation technique. It may be possible to use an activated sludge system, trickling filter or rotating biological contactors which would have the potential to remove and detoxify agricultural as well as industrial wastewater contaminated with Se.



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